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Multi-year analysis of stock composition of a loggerhead turtle (*Caretta caretta*) foraging habitat using maximum likelihood and Bayesian methods

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Abstract

Genetic markers have proven useful for determining which sea turtle rookeries contribute to a particular feeding ground. This information is especially relevant when management concerns include anthropogenic mortality of feeding cohorts, and the suspected presence of endangered populations. One such feeding habitat is the Pamlico–Albemarle Estuarine Complex in North Carolina, which was established as an index area in 1995 to monitor population-specific recovery of sea turtles. Pound nets in the study area were surveyed at random from September–December (1995–1997) to enumerate incidental captures of sea turtles as an index of sea turtle abundance. In this study, we estimated the rookery origins of this feeding cohort using both maximum likelihood and Bayesian based stock analysis programs and compare and contrast these different methodologies. The Bayesian methods appear to yield more realistic estimates of percent contribution to the feeding cohort when information regarding relative population sizes was used. Subsequently, we tested for temporal variation in the frequency of mitochondrial DNA haplotypes and resulting estimates of contribution over a 3-year time span. Mixed stock analysis of the combined data indicated that 80% of the individuals originated from the south Florida nesting population, 12% were from the northeast Florida to North Carolina nesting population, 6% from Yucatan, Mexico, and 2% from other rookeries. Although statistically significant shifts in haplotype frequencies were not observed between the three annual sampling periods, estimates of composition indicated subtle differences in the contributions to this foraging area over the sampling period.

Introduction

The loggerhead turtle, *Caretta caretta*, is a globally distributed species that occupies coastal and pelagic habitats. Although highly adapted for a marine life, females must go ashore to lay their eggs. After emerging from the nest, hatchling turtles enter the ocean and engage in a swimming frenzy that may exceed 24 h (Deraniyagala 1939; Carr and Ogren 1960). The entire life span of marine turtles carries them through spatially and compositionally varying developmental habitats. Upon sexual maturation the cycle is completed with a return of reproductive animals to their natal beach (Carr et al. 1978; Bowen et al, 1992; Fitz-Simmons et al. 1997). Although this generalized

life history cycle has been posited, studies regarding behavior and migrations of benthic immature stages are infrequent.

Loggerhead turtles are abundant in coastal areas of the Northwest Atlantic and the largest nesting population is located in the southeastern United States, with approximately 50,000–85,000 clutches each year (Turtle Expert Working Group 1998). Although protected by legislation, loggerheads face threats from several coastal and offshore fisheries (National Research Council 1990). Protection on beaches is crucial, yet it has become evident that protecting nesting individuals and nests is not enough to prevent the decline of loggerhead populations. Protection of other life history stages is critical to the survival of marine turtle populations (Crouse et al. 1987; Crowder et al. 1994; Heppell et al. 1996; Heppell et al. 2003); therefore, wildlife managers have to consider the demography and temporal dynamics of in-water aggregations when developing management plans for a given population. Demographic information from only one year may be misleading and result in unfounded conclusions. For example, while examining trends in green turtle nesting around Australia, Limpus and Nicholls (1988) detected extreme fluctuations in annual nesting numbers. If only annual data over a short interval are considered, a misleading picture of population decline is apparent. However, when longer intervals (decadal scale) were considered, the large fluctuations in annual nesting numbers indicate a stable population that includes late maturing animals. This study clearly illustrates the limitation of short-term studies of population trends in long-lived species.

Recognizing the need for long-term in-water studies of marine turtles, scientists and managers have begun identifying index abundance areas for intensive monitoring. The Pamlico–Albemarle Estuarine complex, located along the coast of North Carolina, has relatively large loggerhead aggregations within inshore waters from May to December (Epperly et al. 1995). The steady utilization of this area by multiple species of marine turtles and access to the animals through local pound net fisherman make this area an excellent location for long-term monitoring. Long-term monitoring has several goals including: (1) tracking the status and condition of turtles, (2) obtaining data to support stock assessment and trend analyses and (3) providing life history information (Epperly and Braun 1998). One means of supporting the long-term monitoring is through a combination of field and laboratory methodologies that allow researchers to determine the populations affected by management decisions.

Mixed stock analysis (MSA) was developed to monitor the success of salmon management programs in the northwestern United States (Grant et al. 1980). MSA allows researchers to estimate the most likely source populations for an aggregation of individuals using the frequencies of genetic and/or morphological characters (Pella and Milner 1987). Most of the earlier versions of MSA programs were grounded in a maximum likelihood (ML) framework, however, other methods such as Bayesian based algorithms have recently been applied to mixed stock analysis (Pella and Masuda 2001). The ML approach has been applied to multiple MSA studies of marine turtle foraging grounds. Bowen et al. (1996) used MSA of mitochondrial (mtDNA) markers in the hawksbill, *Eretmochelys imbricata*, to determine which nesting populations may be impacted by human induced mortality in a feeding habitat. Other studies have significantly contributed to our understanding of the migratory behavior of loggerhead turtles in the Pacific (Bowen et al. 1995) and the eastern Atlantic (Bolten et al. 1998). More recently Engstrom et al. (2002) examined the composition of a tropical developmental habitat in Panama. In green turtles, *Chelonia mydas*, it was shown that geographically distinct foraging aggregations differ in the relative contributions from source populations (Bass et al. 1998; Lahanas et al. 1998; Bass and Witzell 2000). Effective management, however, not only requires an understanding of spatial variation in foraging locations, but also temporal changes in composition of foraging aggregations over several scales. Genetic markers have been successful in detecting temporal variation in stock composition of fish, such as Dolly varden, *Salvelinus malma* (Krueger et al. 1999). Similarly, genetic markers in marine turtles can be used to detect changes in composition of foraging cohorts. Here we present results from the genetic analysis of three consecutive sampling periods (1995–1997) for juvenile loggerheads in the Pamlico–Albemarle Estuarine complex. Investigations of the foraging aggregation allow us to test for temporal variation in mtDNA haplotype frequencies and to infer corresponding changes in foraging ground composition.

Methods

Throughout the months of September–December during 1995 (n = 114), 1996 (n = 155) and 1997 (n = 156), blood samples and carapace length measurements were collected from benthic immature loggerheads (36–100 cm SCL) caught by pound net fisherman in the Core, eastern Pamlico, and Albemarle Sounds, North Carolina (Figure 1). Approximately 1 ml of blood was placed in 9 ml of lysis buffer (100 mM Tris–HCL, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; pH 8.0) and stored at room temperature. As part of an ongoing study, animals were tagged and released. DNA was isolated from blood samples with the phenol/chloroform method described by Hillis et al. (1996).

A 380 base-pair fragment of the control region of the mitochondrial (mtDNA) genome was amplified with polymerase chain reaction (PCR) methodology (Mullis and Faloona 1987) using the primers TCR-5 and TCR-6 of Norman et al. (1994) and standard reaction conditions (Saiki et al. 1988). Cycle sequencing was conducted with an ABI Prism kit at the University of Florida DNA Sequencing Core and analyzed with an automated DNA sequencer (Applied Biosystems model 373A). Sequences were compared to known loggerhead haplotypes (Encalada et al. 1998; Laurent et al. 1998; Pearce 2001) and assigned a haplotype designation. Sequences that did not match known haplotypes were sequenced in the opposite direction to confirm the accuracy of the initial base calls. New haplotype sequences were deposited in GenBank.

To assess temporal and size class variation in haplotype frequencies, we conducted X^2-tests of independence (Sokal and Rohlf 1981) with the Monte Carlo randomization method in the program CHIRXC (Zaykin and Pudovkin 1993). To correct for multiple tests that resulted in significant P values, the sequential Bonferroni method was employed (Rice 1989). To test for differences in size class frequencies between years, a Kolmogorov–Smirnov 2-sample test was conducted (Sokal and Rohlf 1981) utilizing the NPAR1WAY procedure (SAS Institute, Cary, NC).

Regional rookeries and their associated haplotype frequencies are defined as in Encalada et al. (1998), Laurent et al. (1998) and Pearce (2001) and consisted of: northwest Florida (NWFL), south Florida (SFL), northeast Florida to North Carolina

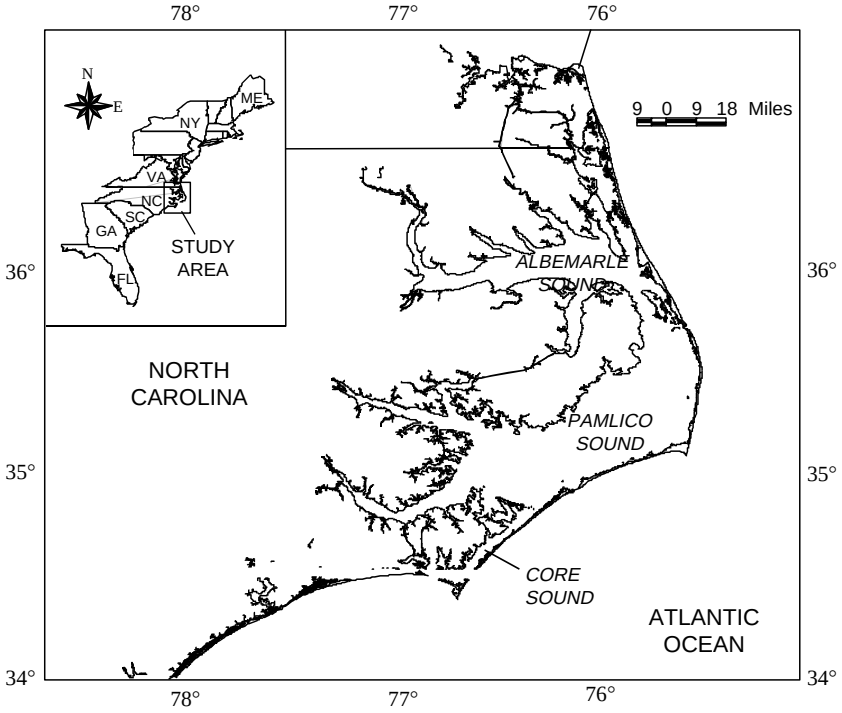

Figure 1. Location of the abundance index area in the Pamlico–Albemarle Estuarine Complex, North Carolina, USA.

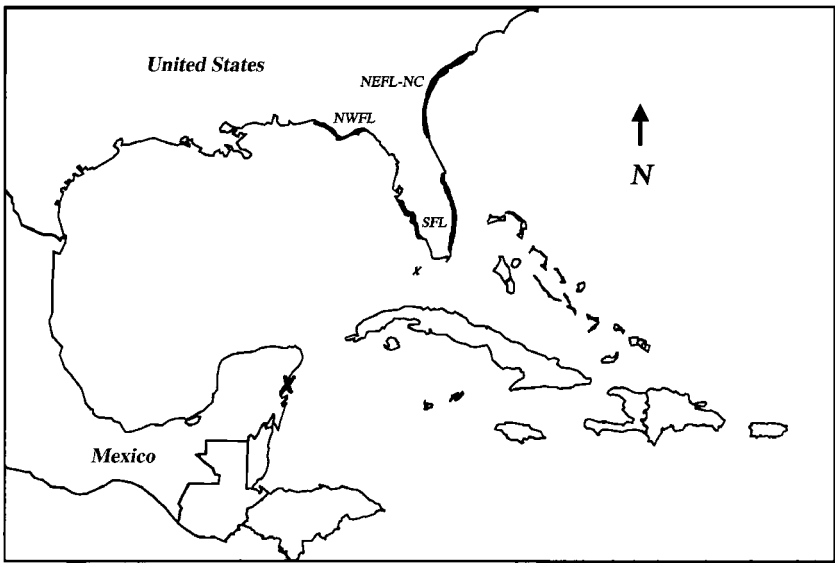

Figure 2. Location of source populations used in the mixed stock analyses. Nesting locations in Brazil, Greece and Turkey are not indicated due to scale. The "X" on the Caribbean coast of the Yucatan peninsula indicates the approximate location of the Mexican nesting population samples and the smaller "X" southwest of the tip of Florida indicates the approximate location of the Dry Tortugas.

(NEFL-NC), Dry Tortugas, Mexico, Brazil, Greece and Turkey (Figure 2). Conventional estimates of F_{ST} and associated P values (from haplotype frequencies and 1000 permutations) between the regional rookeries were calculated using Arlequin (version 2.001; Schneider et al. 2001). Additionally, exact tests of differentiation between all pairs of samples based on haplotype frequencies were conducted using Arlequin (version 2.001; Schneider et al. 2001).

Maximum likelihood (ML) estimates of contributions from surveyed rookeries were obtained using the program SPAM (v.3.5; Alaska Department of Fish and Game 2001). SPAM employs two algorithms to estimate the stock composition of a random sample of a mixture from data that consists of the observed genotypic frequencies of the mixture and the observed genotypic frequencies in source populations. The iteratively re-weighted least squares (IRLS) algorithm computes a conditional maximum likelihood estimate of composition using modified weights along with the composition vector from one iteration to the next. An EM algorithm is employed to constrain estimates such that the likelihood function is non-decreasing during the search (Pella and Milner 1987). All source populations (regional rookeries) were included in the analyses. Initial estimates were generated assuming that all source populations contributed with equal probabilities and other starting points were also used (Reynolds 2001). An assumption of SPAM is that the haplotypes observed in the random sample have been identified in source populations; therefore haplotypes not observed previously in the source populations were not included in the ML analyses. Individuals that were recaptured during subsequent years were included in the individual year analysis, but not in the combined analysis of all three years. Adjusted sample sizes for the ML analysis are shown in Table 2.

A Bayesian approach was also used to estimate foraging ground composition using the same data as described above. BAYES (Pella and Masuda 2001) incorporates information from the observed data (stock and mixture) to estimate relative stock contributions. Unlike the ML approach, BAYES is reportedly not biased by the presence of rare haplotypes, such as singletons or those that occur at $< 5\%$ (Pella and Masuda 2001). This is particularly advantageous in the analysis of turtle mtDNA control region data, which is characterized by many rare haplotypes in source populations and mixed

Table 1. Source populations used in both the Maximum Likelihood and Bayesian analyses. Mediterranean data and alternative haplotype designations in parentheses are from Laurent et al (1998). Additional data for NWFL, SFL and the Dry Tortugas are from Pearce (2001). The remaining individuals are from Encalada et al. (1998). Population size estimates are derived from Ehrhart et al. (2003) and Margaritoulis et al. (2003)

Haplotype	NWFL	SFL	NEFL-NC	Dry Tortugas	Mexico	Greece	Turkey	Brazil
CC-A1 (C1)	38	52	104	4				
CC-A2 (A1)	7	45	1	50	11	78	19	
CC-A3 (A3)	2	4			2		13	
CC-A4								11
CC-A5		1						
CC-A6						2		
CC-A7 (A7)	2	3						
CC-A8					1			
CC-A9 (A4)				2	1			
CC-A10 (A2)				2	5	1		
CC-A11		1						
CC-A14 (C3)		2						
CC-A20		1						
Total	49	109	105	58	20	81	32	11
Rookery Size	600	67,100	6200	217	1800	3660	1366	2400

aggregations. In addition the Bayesian approach allows for the incorporation of informed priors: starting points for iterations approaching the optimal solution. Here we used three approaches in setting the prior parameters of the posterior distribution: equal contribution from each stock (BM1), zero contribution (BM2) and contribution weighted by population size (BM3). Size estimates of the nesting populations were taken from Ehrhart et al. (2003) and Margaritoulis et al. (2003). The mean, standard deviation and equal-tail bounds of the posterior intervals were recorded for each approach. The most likely model under the Bayesian approach was determined by examination of the spread of the equal-tail bounds of the posterior intervals, which are similar to the 95% confidence intervals of the maximum likelihood approach. The Gelman-Rubin shrink factor was also used to test for anomalous realizations of the Bayes predictive posterior distribution (Pella and Masuda 2001). Values greater than 1.20 indicated a lack of convergence in the algorithm and the corresponding estimates were considered unreliable. We explored the use of multiple hypothesis testing in Bayesian methods such as the Bayes factor (Kass and Raferty 1995), however, the results and output of the BAYES program were not amenable to these tests (Pella and Masuda 2001).

Results

Mitochondrial DNA analyses

The estimated haplotype composition and size of source populations are listed in Table 1. Population pair wise F_{ST} values ranged from 0.0415 to 0.9828 and all were highly significant ($P \leq 0.005$; Table 2). Exact tests of differentiation were also significant ($P \leq 0.05$) for all source populations (data not shown).

Of the 300 randomly selected blood samples, 295 produced readable sequences. The overall frequency of haplotypes between the 3 years was very similar (Table 3). The majority of animals surveyed ($n = 295$) possessed either haplotype CC-A1 (56%) or haplotype CC-A2 (33%). Both haplotypes are found in multiple nesting locations in the Atlantic. Other haplotypes observed that have been identified from a nesting location were CC-A3, CC-A4, CC-A5, CC-A7, CC-A8, CC-A9, CC-A10, and CC-A14 (Encalada et al. 1998; Pearce 2001, http://accstr.ufl.edu/ccmtdna.html). Haplotype CC-A13 was observed among the

Table 2. Population subdivision estimates for source populations used in the mixed stock analysis. Above the diagonal: F_{ST} values. Below the diagonal: + indicates significant P values

	NWFL	SFL	NEFL-NC	Dry Tortugas	Mexico	Greece	Turkey	Brazil
NWFL	–	0.1204	0.1913	0.6176	0.4679	0.7709	0.5175	0.7151
SFL	+	–	0.3999	0.2716	0.1878	0.4098	0.2439	0.5486
NEFL-NC	+	+	–	0.8851	0.8518	0.9570	0.8517	0.9828
Dry Tortugas	+	+	+	–	0.1628	0.0415	0.2509	0.7998
Mexico	+	+	+	+	–	0.3857	0.0958	0.6068
Greece	+	+	+	+	+	–	0.4339	0.0369
Turkey	+	+	+	+	+	+	–	0.6615
Brazil	+	+	+	+	+	+	+	–

Table 3. Annual haplotype composition for the North Carolina foraging ground population and the nesting or foraging aggregation the haplotype has been observed in. Numbers in parentheses represent the number of individuals used in the mixed stock analysis. Individuals that possessed haplotypes not identified in a source population were not included in the mixed stock analysis. Numbers in parentheses for haplotypes CC-A1, CC-A2, and CC-A3 are the actual frequency of those haplotypes observed per year plus recaptures. Total numbers in parentheses represent the sample size used in the annual and combined maximum likelihood analyses. Recaptures were included only once in the combined analysis to prevent duplications of single individuals

Haplotype	1995	1996	1997	Combined	Source Populations
CC-A1	55	58 (60)	52 (54)	165	NWFL, SFL, NEFL-NC
CC-A2	37	31 (32)	30 (33)	98	NWFL, SFL, NEFL-NC, Mexico, Greece
CC-A3	2	3 (4)	3	8	NWFL, SFL, Mexico
CC-A4	1	0	0	1	Brazil
CC-A5	0	1	2	3	SFL
CC-A7	1	3	1	5	NWFL, SFL
CC-A8	0	0	1	1	Mexico
CC-A9	0	0	1	1	Mexico
CC-A10	1	0	3	4	Mexico
CC-A13	0	0	1	1	*Azores Foraging*
CC-A14	0	3	4	7	SFL
CC-A18	0	0	1	1	*North Carolina Foraging*
Total	97	99 (103)	99 (102)	295 (293)	

foraging ground samples, but the rookery origin of this haplotype has not been determined although it has been observed in samples from other foraging locations (Bolten et al. 1998). One new haplotype was found in a single 1997 sample (CC-A18; AY508983). Haplotype (h) and nucleotide (π) diversities, respectively, were 0.5378 ± 0.0288 and 0.0269 ± 0.0137 in 1995, 0.5615 ± 0.0379 and 0.0265 ± 0.0135 in 1996, and 0.6343 ± 0.0377 and 0.0278 ± 0.0141 in 1997. In 1997 the number of haplotypes increased to 11 resulting in increased h and π.

Haplotype frequencies were not significantly different between the three years (1995 versus 1996, $X^2 = 8.07$, $P = 0.296$; 1995 versus 1997, $X^2 = 12.74$, $P = 0.245$; 1996 versus 1997, $X^2 = 8.76$, $P = 0.651$). In addition there were no significant differences in size class composition for the three years (1995 versus 1996, $P = 0.5159$; 1995 versus 1997, $P = 0.2378$; 1996 versus 1997, $P = 0.9768$), or in the haplotype frequencies among the size classes ($X^2 = 85.05$, $P = 0.707$).

Mixed stock analysis

The results of the maximum likelihood (ML) and Bayesian analyses for the individual years and the three years combined are presented in Table 4.

The ML estimate and the Bayesian estimate where the prior was set to equal probability of contributing (BM1) consistently gave similar results for the three years and for the combination of the individual years. These results were characterized by large spreads and overlap among the source populations in terms of the confidence intervals (Table 4). Examination of the mean estimates for both the ML and BM1 analyses indicate that all stocks are contributing. The exception to the assignment of contribution by all candidate contributors is found in the ML estimates for 1996–1997 where Brazil is not allocated any portion of the contribution. This is in contrast to BM1 where Brazil is allocated a tiny portion (mean = 0.0013; Conf. Int. = 0.0000–0.0122) in both years. The BM1 results are deemed unreliable for the following reasons. All Gelman-Rubin shrink factor estimates for potential contributors were ≥1.2 indicating lack of convergence in the MCMC chains. Qualitatively, the results are unreliable because Brazil is essentially fixed for haplotype D (Table 1) and no individuals carrying this haplotype were identified during the 1996–1997 sample periods. Overall both the ML and BM1 estimates appear to be inflated. For example, in 1995, 1996 and in the combined years estimates, the contribution of NWFL is consistently large and in some cases larger than the contributions from SFL and NEFL-NC. This is extremely unlikely as the nesting population in NWFL is very small relative to SFL (600 versus 61700; Ehrhart et al. 2003). The small size of the population coupled with the distance from the foraging ground location make these results biologically unrealistic. The large confidence intervals and standard errors (not shown) for ML results provide no reliable resolution of stock contribution with our dataset. We reject the results from both the ML and BM1 analyses as being unreliable estimates for these reasons in addition to the following results for the other models assessed.

In contrast, Gelman-Rubin shrink factor estimates for the Bayesian model with priors set to zero (BM2) and priors set to proportion of population size estimates (BM3) were all equal to 1.0 indicating convergence in the MCMC chains. In addition the confidence intervals about the mean estimates were much tighter indicating less variation in the MCMC chain estimates. The stock contribution estimates for 1995 again were biologically problematic and we believe this is due to the presence of rare haplotypes in the foraging ground sample. Both the ML and Bayesian methodologies have problems with rare haplotypes with SPAM overestimating the contribution of individual stocks where the rare haplotype is found. While the Bayesian analysis is an improvement on these analytical problems, the use of the Dirichlet probability density for the baseline prior in the stocks still results in an overestimation of the contribution of NWFL in 1995 (BM2; mean = 0.500; Conf. Int. = 0.000–1.000). In BM2 the baseline prior parameters were determined solely by the pseudo-Bayes method and the haplotype composition of the stocks. In other words, we included no other information about the stocks such as population size or distance from the foraging ground. In addition to other criteria, the pseudo-Bayes method was used to minimize the effect of large variation in loci frequencies among stocks (Pella and Masuda 2001). While these priors are assumed to have no affect or to be weakly informative, we believe that they still result in the overestimation of contribution by individual stocks when coupled with low variation in relative frequencies among other haplotypes. For example, the haplotype composition for the NWFL and SFL stocks exhibits low variation in terms of the relative frequencies of shared haplotypes CC-A1, CC-A3 and CC-A7, however, rare haplotypes restricted to SFL (CC-A5, CC-A11, CC-A14 and CC-A20) also exhibit low variation (0 versus 1 for CC-A5) (Table 1). In an attempt to minimize the affect of unsampled or ''missed'' rare haplotypes this method results in ''corrections'' that bias the estimated contributions of stocks that do not possess one of the rare haplotypes but exhibit similar relative frequencies among shared observed haplotypes. Potentially, this problem can be resolved in our situation by two methods: (1) significantly increase the sampling of the stock populations and/or (2) use of a more informative prior. We opted for the 2nd method. When the prior for the stock mixture proportions is set to nesting population size for all years and the combined dataset, the estimate seems more reasonable based on the reproductive potential of the nesting population. We acknowledge that we are ''choosing'' a method of analysis that appears to generate results that coincide more with our biological knowledge of the nesting populations and

Table 4. Maximum Likelihood (ML) and Bayesian estimates of stock composition for the individual years (1995, 1996 and 1997) and the combined data for the N.C. loggerhead foraging ground. The mean estimates of stock composition and the 2.5% and 97.5% confidence intervals surrounding these mean estimates are shown. BM1 is the Bayesian analysis with the prior set to an equal probability of contribution by all stocks. BM2 is the Bayesian analysis with the prior set to zero and in BM3 the prior is set to reflect the individual stock's proportion of estimated nesting population in the Atlantic and Mediterranean

	ML		BM1	
	Mean	97.50%	Mean	97.50%
1995				
NWFL	0.2123	0.0000–0.7508	0.4391	0.0000–0.8386
SFL	0.0346	0.0000–0.5309	0.1201	0.0000–0.8342
NEFL/NC	0.3795	0.0002–0.6602	0.1545	0.0000–0.5498
MEXICO	0.0145	0.0000–0.1033	0.0159	0.0000–0.1146
Dry Tortugas	0.1122	0.0000–0.4651	0.1278	0.0000–0.4108
GREECE	0.2010	0.0000–0.4413	0.1218	0.0000–0.3839
TURKEY	0.0344	0.0000–0.1438	0.0136	0.0000–0.1017
BRAZIL	0.0114	0.0000–0.0412	0.0071	0.0000–0.0365
1996				
NWFL	0.2136	0.0000–0.7370	0.1631	0.0000–0.5947
SFL	0.5203	0.0005–0.9546	0.7284	0.3245–0.9956
NEFL/NC	0.1726	0.0000–0.5114	0.0729	0.0000–0.3574
MEXICO	0.0000	0.0000–0.0001	0.0042	0.0000–0.0386
Dry Tortugas	0.0092	0.0000–0.1658	0.0097	0.0000–0.0874
GREECE	0.0369	0.0000–0.2367	0.0084	0.0000–0.0754
TURKEY	0.0398	0.0000–0.1773	0.0120	0.0000–0.0856
BRAZIL	0.0000	0.0000–0.0001	0.0013	0.0000–0.0121
1997				
NWFL	0.0624	0.0000–0.4618	0.0692	0.0000–0.4338
SFL	0.4719	0.0006–0.8603	0.6636	0.3084–0.9233
NEFL/NC	0.2503	0.0001–0.5265	0.1139	0.0000–0.3780
MEXICO	0.0949	0.0000–0.2421	0.1126	0.0293–0.2344
Dry Tortugas	0.0704	0.0001–0.3142	0.0242	0.0000–0.1730
GREECE	0.0163	0.0000–0.1990	0.0091	0.0000–0.0847
TURKEY	0.0177	0.0000–0.1144	0.0062	0.0000–0.0518
BRAZIL	0.0000	0.0000–0.0003	0.0013	0.0000–0.0122
Combined				
NWFL	0.1284	0.0000–0.5735	0.1243	0.0000–0.4575
SFL	0.4591	0.0002–0.8460	0.6671	0.3763–0.9456
NEFL/NC	0.2398	0.0001–0.4805	0.1169	0.0000–0.3682
MEXICO	0.0397	0.0000–0.1204	0.0541	0.0106–0.1273
Dry Tortugas	0.0763	0.0000–0.2809	0.0216	0.0000–0.1379
GREECE	0.0299	0.0000–0.2258	0.0087	0.0000–0.0733
TURKEY	0.0153	0.0000–0.0814	0.0055	0.0000–0.0453
BRAZIL	0.0034	0.0000–0.0137	0.0019	0.0000–0.0112
	BM2		BM3	
1995				
NWFL	0.5000	0.0000–1.0000	0.0061	0.0000–0.0373
SFL	0.2500	00000–1.0000	0.8321	0.2204–1.0000
NEFL/NC	0.1444	0.0000–0.6519	0.1045	0.0000–0.4806
MEXICO	–	–	0.0121	0.0000–0.0934

Table 4. Continued

	ML		BM1	
	Mean	97.50%	Mean	97.50%
Dry Tortugas	0.0551	0.0000–0.4904	0.0034	0.0000–0.0238
GREECE	0.0505	0.0000–0.4487	0.0368	0.0000–0.2911
TURKEY	–	–	0.0012	0.0000–0.0121
BRAZIL	–	–	0.0039	0.0000–0.0301
1996				
NWFL	–	–	0.0033	0.0000–0.0220
SFL	1.0000	1.0000–1.0000	0.9302	0.6494–1.0000
NEFL/NC	–	–	0.0606	0.0000–0.3311
MEXICO	–	–	0.0004	0.0000–0.0043
Dry Tortugas	–	–	0.0003	–
GREECE	–	–	0.0033	0.0000–0.0425
TURKEY	–	–	0.0016	0.0000–0.0222
BRAZIL	–	–	0.0003	0.0000–0.0034
1997				
NWFL	–	–	0.0036	0.0000–0.0229
SFL	0.8969	0.7938-0.9661	0.8128	0.4656–0.9602
NEFL/NC	–	–	0.0725	0.0000–0.3535
MEXICO	0.1031	0.0339-0.2062	0.1068	0.0324–0.2241
Dry Tortugas	–	–	0.0003	–
GREECE	–	–	0.0030	0.0000-0.0339
TURKEY	–	–	0.0007	0.0000-0.0065
BRAZIL	–	–	0.0003	0.0000-0.0032
Combined				
NWFL	–	–	0.0098	0.0000-0.1685
SFL	0.9467	0.8891–0.9825	0.8061	0.5376-0.9746
NEFL/NC	–	–	0.1219	0.0000-0.3491
MEXICO	0.0533	0.0175–0.1109	0.0578	0.0174-0.1279
Dry Tortugas	–	–	0.0004	–
GREECE	–	–	0.0026	0.0000–0.0306
TURKEY	–	–	0.0007	0.0000–0.0072
BRAZIL	–	–	0.0007	0.0000–0.0071

behavior of marine turtles. As a compromise we suggest that BM2 is a conservative estimate of the stock contributions to the NC foraging ground, however, we will limit further discussion regarding stock composition to the results from BM3.

Individuals from the SFL nesting population dominated the foraging ground aggregation during all three years (83%, 93% and 81% in 1995, 1996, and 1997, respectively; Table 4). NEFL-NC and Mexico were the second largest contributors with a range in composition over the three years of 6–10% and 0–10%, respectively. The only other contribution estimate greater than 0 was that of Greece at 3% in 1995 (Table 4). SFL, NEFL-NC and Mexico were indicated as contributors when data from all three years were combined; 80%, 12% and 6%, respectively. The remaining source populations contributed 2% to the NC foraging aggregation.

Discussion

We determined that the Bayesian analysis (BM3) provided the best performance in resolving contributions to the combined annual samples from

the Pamlico–Albemarle Estuarine complex. In the combined analysis (n = 293), the majority of juveniles and sub-adults utilizing this habitat originate from the SFL nesting population (80%). The second largest contributor is the smaller NEFL-NC nesting population (12%) with contributions from rookeries in Mexico (6%) and other rookeries (2%). The results for individual years exhibit a similar trend with the majority of individuals originating from the southern Florida nesting population and smaller contributions from nesting locations outside of the United States. The findings reported here are consistent with those of other researchers who have noted a correlation between the size of loggerhead nesting populations and their relative contributions (Bolten et al. 1998; Rankin-Baransky et al. 2001).

Bolten et al. (1998) investigated the composition of pelagic loggerheads in the vicinity of the Azores and Madeira in the eastern Atlantic. This study detected contributions from three nesting populations in the western Atlantic: SFL, 71%; NEFL-NC, 19%; Mexico, 11%. They found no difference in the haplotype composition among size classes, with individual sizes ranging from 9 cm to 71 cm (CCL) in the Azores and 20–55 cm (CCL) in Madeira. Samples from loggerheads stranded along the northeast coast of the United States indicated that 59% originated from the SFL nesting population, 25% from the NEFL-NC population, and 16% from Mexico (Rankin–Baransky et al. 2001). The size of these individuals ranged from 39 cm to 109 cm (mean = 54 cm SCL). They concluded that although the stranded loggerheads in the northeast were members of the same genetic populations as those surveyed by Bolten et al. (1998), the northeastern United States individuals represent a different developmental stage. There may be two immature life-history phases: an early pelagic phase of hatchlings, post-hatchlings and small juveniles and a benthic sub-adult phase that reaches maturity in shallow coastal waters (Carr 1987; Limpus et al. 1994). The loggerheads utilizing the western Atlantic coastal areas are representative of the benthic sub-adult phase prior to recruitment to nesting beaches in the western Atlantic (Dodd 1988; Laurent et al. 1998; Rankin-Baransky et al. 2001). The mixed stock analysis results for the N.C. foraging location support these findings and most likely represent the benthic immature phase. Consistent with Rankin–Baransky et al. (2001), the size of individuals sampled in the N.C. foraging location ranged from 36 cm to 100 cm (SCL). The results presented here are different from the northeastern Atlantic foraging area, but we cannot determine how much of that difference is due to the analytical methods. The higher percentage of the SFL animals in the N.C. foraging location could be a function of the distance from the nesting beaches in Florida. If animals don't make a trans-Atlantic trip, they may spend their developmental history moving up and then back down the eastern seaboard of the United States. Mixed stock analysis of loggerhead strandings along the eastern seaboard may provide an indication of this type of movement (Bowen et al. *Submitted*).

Several researchers have suggested that juvenile turtles preferentially recruit to foraging grounds which are located near their nesting beaches (Laurent et al. 1998; Engstrom et al. 2002; Bass and Witzell 2000). Although the NEFL-NC nesting population does not dominate the composition of the N.C. foraging location, there does appear to be a larger proportion from this rookery relative to expectations based on rookery size. This provides circumstantial evidence that benthic sub-adult turtles are preferentially recruiting to foraging locations proximate to their natal origin. However, influences such as differential survival of offspring from nesting populations, currents and seasonal migratory movements complicate a simple model of loggerhead foraging location composition based on the relative sizes and/or location of the nesting assemblages.

In terms of temporal variation at foraging locations, we did not observe statistically significant variation in the size classes of individuals over the three years, the haplotype frequencies of the size classes nor in the annual haplotype frequencies. Due to the large confidence intervals about the mean estimates we cannot conclude that there are statistically significant differences among the annual estimates of contribution, either. There are several potential explanations that can be categorized as biological or statistical in nature. The biological explanation is that temporal variation in composition is not a characteristic of marine turtle foraging ground populations. We reject this explanation because we do not believe that these populations are static. Recruitment is a dynamic and continuous process and subject to differential

survival throughout their life history and across nesting colonies. Although animals may show a preference for a location, the probability of changes at the nesting location or changes in oceanic currents not being reflected on these foraging grounds intuitively seems low.

The statistical explanation encompasses various levels and some biological aspects. The first deals with the sampling design and the second with constraints of the analyses. First, the sampling period (3 years) may not be long enough to detect temporal variation. Loggerheads are long-lived animals and changes in the productivity of nesting locations may need greater time periods for detection. Second, although the nesting populations included here are significantly different in terms of their haplotype frequencies, there still may not be enough variation for the ML or Bayesian methods to generate consistent and reasonable estimates (Epifanio et al. 1995). One of our main problems with the results from these analyses is trying to derive trends based on inconsistent characteristics of the estimates. For example, the estimates for BM2 exhibit little variation within years for 1996 and 1997. The BM2-1995 estimate is problematic with biologically unrealistic contributions from NWFL, a correspondingly low contribution from SFL and large confidence intervals about the estimates. Moreover the estimates do not reflect the qualitative interpretation of the presence of animals from Brazil and Mexico (CC-A4 and CC-A10) for both 1995 and 1996. BM2 does indicate significant temporal variation in foraging ground composition between 1996 and 1997, but to accept these estimates we have to conclude that a minimum of 1 and maximum of 2 nesting populations are contributing individuals. This suggestion of only one stock contributing individuals seems overtly conservative and again biologically unrealistic. Do we mix and match analytical methods and assumptions? At this point we are not prepared to do that. Consequently, we cannot make a definite conclusion on temporal variation.

The mean estimates of contribution from BM3 between individual years are different (albeit not statistically significant) among the three years, revealing an increase in the percentage of individuals from the nesting population in Quintana Roo, Mexico. These results are not surprising due to the presence of haplotypes CC-A8 and CC-A9 and the increase in number of individuals possessing haplotype CC-A10. All three of these haplotypes have only been detected in the Mexico nesting population (Encalada et al. 1998). These changes in haplotype composition are reflected in the diversity indices (haplotype and nucleotide diversities) and in the BM3 estimates.

If we accept the mean estimates indicating conservative changes in relative frequencies of stock composition, then multiple factors could result in the annual differences in contributions from the nesting populations. Changes in the number or survivorship of hatchlings produced at the nesting localities could result in changes in the relative proportions of individuals from these nesting localities foraging in N.C. A long-term study of the loggerhead nesting population at Cumberland Island, Georgia has detected variation in both the frequency and size of clutches (Frazer and Richardson 1985). An 11-year study of hawksbills nesting in Antigua provided evidence for significant variation in seasonal emergence success of hatchlings (Richardson et al. 2000). In this case, hurricanes had a significant impact on the survivorship of hatchlings. It may be possible that conservation efforts in Mexico have been successful in increasing the survivorship of multiple cohorts of animals as evidenced here in the increase in 1997 of animals originating from Mexico. With increases in population size, the sub-adult animals in Mexico may not be able to find suitable or sufficient quantities of foraging habitat near their natal beach causing travel further away from their location of origin. Other factors that could result in temporal variation in foraging ground composition include seasonal variation and sampling regimes and seasonal changes in currents and other climatic factors.

The eastern seaboard of the United States appears to be an important developmental habitat for sub-adult loggerhead turtles originating from rookeries in the Southeastern United States (Dodd 1988). This study indicates that there may be variation in the composition of foraging grounds as evidenced by the changes in composition from 1995 to 1997 and illustrates some potential pitfalls of ML and Bayesian analyses. We strongly believe that the ability to detect variation in the composition of foraging grounds is an essential tool for monitoring changes in sea turtle populations and should be explored more fully. Without the ability

to track hatchlings from the time they leave the beach until they reach sexual maturity, we must rely on indirect methods to assess the status of populations. Identification of the stocks that utilize shared foraging grounds and quantification of the numbers of individuals originating from those stocks can be used as an indicator of the success of protective measures enforced on beaches and more importantly in the water. While the methods used in this study lack some power and require careful interpretation, they do provide an indication of changes in stock estimates that will be useful in determining monitoring strategies. Although these changes are not statistically significant, we are mindful that as demonstrated by Limpus and Nicholls (1988), short-term monitoring (in this study, ≤3 years) may be insufficient in detecting significant changes in the demography of long-lived species. The relatively large number of individuals originating from Mexico also emphasizes the necessity for international cooperation in the management of loggerheads in the Atlantic.

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References

Alaska Department of Fish and Game (2001) *SPAM Version 3.5: Statistics Program for Analyzing Mixtures*. Alaska Department of Fish and Game, Commercial Fisheries Division, Gene Conservation Lab, Anchorage, Alaska. Available for download from: http://www.cf.adfg.state.ak.us/geninfo/research/genetics/software/spampage.htm.

Bass AL, Lagueux CJ, Bowen BW (1998) Origin of green turtles, *Chelonia mydas*, at "sleeping rocks" off the northeast coast of Nicaragua. *Copeia*, **4**, 1064–1069.

Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast: evidence from mtDNA markers. *Herpetologica*, **3**, 357–367.

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada SE, Bowen BW (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecol. Appl.*, **1**, 1–7.

Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ (1995) Trans-Pacific migrations of the loggerhead sea turtle demonstrated with mitochondrial DNA markers. *Proc. Natl. Acad. Sci. USA*, **92**, 3731–3734.

Bowen BW, Bass AL, Garcia–Rodriguez A, Diez CE, van Dam R, Bolten A, Bjorndal KA, Miyamoto MM, Ferl RJ (1996) Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. *Ecol. Appl.*, **2**, 566–572.

Bowen BW, Meylan AB, Ross JP, Limpus CJ, Balazs GH, Avise JC (1992) Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution*, **46**, 865–881.

Carr A (1987) New perspectives on the pelagic stage of sea turtle development. *Conserv. Biol.*, **1**, 103–121.

Carr AF, Carr M, Meylan AB (1978) The ecology and migrations of sea turtles. 7. The west Caribbean green turtle colony. *Bull. Am. Mus. Nat. Hist.*, **162**, 1–46.

Carr AF, Ogren L (1960) The ecology and migrations of sea turtles. 4. The green turtle in the Caribbean Sea. *Bull. Am. Mus. Nat. Hist.*, **121**, 1–48.

Crouse DT, Crowder LB, Caswell H (1987) A stage-based population model for loggerhead sea turtles and implications for conservation. *Ecology*, **5**, 1412–1423.

Crowder LB, Crouse DT, Heppell SS, Martin TH (1994) Predicting the impact of turtle excluder devices on loggerhead sea turtle populations. *Ecol. Appl.*, **4**, 437–445.

Deraniyagala P (1939) The tetrapod reptiles of Ceylon. Testudinates and crocodilians. *Ceylon J. Sci. (Colombo Museum of Natural History Series)*, **1**, 242.

Dodd CK (1988) Synopsis of the biological data on the loggerhead sea turtle. *Caretta caretta* (Linnaeus 1758). US Fish and Wildl. Serv., Biol. Report 88–14.

Ehrhart LM, Bagley DA, Redfoot WE (2003) Loggerhead turtles in the Atlantic Ocean: geographic distribution, abundance, and population status. In: *Loggerhead Sea Turtles* (eds. Bolten AB, Witherington BE), pp. 157–174. Smithsonian Books, Washington.

Encalada SE, Bjorndal KA, Bolten AB, Zurita JC, Schroeder B, Possardt E, Sears CJ, Bowen BW (1998) Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. *Mar. Biol.*, **130**, 567–575.

Engstrom TN, Meylan PA, Meylan AB (2002) Origin of juvenile loggerhead turtles (*Caretta caretta*) in a tropical developmental habitat in Caribbean Panama. *Animal Conserv.* **5**, 125–133.

Epifanio JM, Smouse PE, Kobak CJ, Brown BL (1995) Mitochondrial DNA divergence among populations of American shad (*Alosa sapidissima*): how much variation is enough for mixed stock analysis? *Can. J. Fish. Aquat. Sci.*, **52**, 1688–1702.

Epperly SP, Braun J (1998) Development of an index of sea turtle abundance in the Pamlico–Albemarle Estuarine complex. In: *Proceedings of the Sixteenth Annual Symposium on Sea Turtle Biology and Conservation*. (compilers Byles R, Fernandez Y), p. 44. NOAA Technical Memorandum NMFS-SEFSC-412.

Epperly SP, Braun J, Veishlow A (1995) Sea turtles in North Carolina waters. *Conserv. Biol.*, **2**, 384–394.

FitzSimmons NN, Goldizen AR, Norman JA, Moritz C, Miller JD, Limpus, CJ (1997) Philopatry of male marine turtles inferred from mitochondrial markers. *Proc. Nat. Acad. Sci. USA*, **94**, 8912–8917.

Frazer NB, Richardson JI (1985) Annual variation in clutch size and frequency for loggerhead sea turtles, *Caretta caretta*, nesting on Little Cumberland Island, Georgia, USA. *Herpetologica*, **41**, 246–251.

Grant WS, Milner GB, Krasnowski P, Utter FM (1980) Use of biochemical genetic variants for identification of sockeye salmon (*Oncorrhynchus nerka*) stocks in Cook Inlet, Alaska. *Can. J. Fish. Aquat. Sci.*, **37**, 1236–1247.

Heppell SS, Crouse DT, Crowder LB. (1996) A model evaluation of headstarting as a management tool for long-lived turtles. *Ecol. Appl.*, **6**, 556–565.

Heppell SS, Snover ML, Crowder LB (2003) Sea turtle population ecology. In: *Biology of Sea Turtles, Volume II* (eds. Lutz PL, Musick JA, Wyneken J), pp. 275–306. CRC Press, Inc., Boca Raton, Florida.

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: Sequencing and cloning. In: *Molecular Systematics* (eds. Hillis DM, Moritz C, Mable BK), pp. 321–381. Sinauer Associates, Sunderland, Massachusetts.

Kass RE, Raferty AE (1995) Bayes Factors. *J. Am. Statist. Assoc.*, **90**, 773–795.

Krueger CC, Wilmot RL, Everett RJ (1999) Stock origins of Dolly Varden collected from Beaufort Sea coastal sites of arctic Alaska and Canada. *Trans. Am. Fish. Soc.*, **128**, 49–57.

Lahanas PN, Bjorndal KA, Bolten AB, Encalada SE, Miyamoto MM, Valverde RA, Bowen BW (1998) Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: Evidence for multiple origins. *Mar. Biol.*, **130**, 345–352.

Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D, Abd El-Mawla EM, Hadoud DA, Gomati HE, Domingo M, Hadjichristophosou M, Kornaraky L, Demirayak F, Gautier CH (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. *Mol. Ecol.*, **7**, 1529–1542.

Limpus CJ, Couper PJ, Read MA (1994) The loggerhead *Caretta caretta* in Queensland: population structure in a warm temperate feeding area. *Memoirs of the Queensland Museum*, **1**, 195–204.

Limpus CJ, Nicholls N (1988) The Southern Oscillation regulates the annual numbers of green turtles, *Chelonia mydas*, breeding around Australia. *Aust. Wildl. Res.*, **15**, 157–162.

Margaritoulis D, Argano R, Baran I, Bentivegna F, Bradai MN, Camiñas JA, Casale P, De Metrio G, Demetropoulos A, Gerosa G, Godley BJ, Hadoud DA, Houghton J, Laurent L, Lazar B (2003) Loggerhead turtles in the Mediterranean Sea: present knowledge and conservation perspectives. In: *Loggerhead Sea Turtles* (eds. Bolten AB, Witherington BE), pp. 175–198. Smithsonian Books, Washington.

Mullis KB, Faloona F (1987) Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.*, **155**, 335–350.

National Research Council (1990) *Decline of the Sea Turtles: Causes and Prevention*. National Academy Press, Washington, DC.

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: Genetic markers for ecological studies of marine turtles. *Mol. Ecol.*, **3**, 363–373.

Pearce AF (2001) *Contrasting population structure of the loggerhead turtle (Caretta caretta) using mitochondrial and nuclear DNA markers*. MS Thesis, University of Florida, Gainesville, Florida.

Pella JJ, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. *Fish. Bull.*, **99**, 151–167.

Pella JJ, Milner GB (1987) Use of genetic marks in stock composition analysis. In: *Population Genetics and Fishery Management* (eds. Ryman N, Utter F), pp. 247–276. University of Washington Press, Seattle.

Rankin-Baransky K, Williams CJ, Bass AL, Bowen BW, Spotila JR (2001) Origin of loggerhead turtles stranded in the northeast Atlantic as determined by mtDNA analysis. *J. Herpetol.*, **35**, 638–646.

Reynolds JH (2001) *SPAM Version 3.5: User's Guide Addendum. Addendum to Special Publication 15*. Alaska Department of Fish and Game, Commercial Fisheries Division, Gene Conservation Lab, Anchorage, Alaska. Available for download from: http://www.cf.adfg.state.ak.us/geninfo/research/genetics/software/spampage.htm.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Richardson JI, Bell R, Richardson TH (2000) Population ecology and demographic implications drawn from an 11-year study of nesting hawksbill turtles, *Eretmochelys imbricata*, at Jumby Bay, Long Island, Antigua, West Indies. *Chel. Conserv. Biol.*, **3**, 244–250.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–491.

Schneider S, Roessli D, Excoffier L (2001) *Arlequin ver 2.001: A software for population genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sokal RR, Rohlf FJ (1981) *Biometry*, 2nd edn. W.H. Freeman and Co., San Francisco, CA.

Turtle Expert Working Group (1998) *An assessment of the Kemp's ridley (Lepidochelys kempii) and loggerhead (Caretta caretta) sea turtle populations in the western North Atlantic*. U.S. Dep. Commer. NOAA Technical Memorandum NMFS-SEFSC-409.

Turtle Expert Working Group (2000) *Assessment update for the Kemp's ridley and loggerhead sea turtle populations in the western North Atlantic*. U.S. Dep. Commer. NOAA Technical Memorandum NMFS-SEFSC-444.

Zaykin DV, Pudovkin AI (1993) Two programs to estimate significance of X^2 values using pseudoprobability tests. *J. Hered.*, **84**, 152.